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GENETIC DIVERSITY OF TWO IMPORTANT GENES FROM SUCKING AGRICULTURAL PEST *APHIS GOSSYPII* (G) POPULATIONS COLLECTED FROM KARNATAKA, INDIA

B. N. CHAITANYA[1], R. ASOKAN[2] & T. SITA[3]

[1,2]Division of Biotechnology, Indian Institute of Horticultural Research (IIHR), Hesaraghatta Lake Post, Bengaluru, India

[3]Head Division of Biotechnology, St. Martin's College of Engineering, Secunderabad, Telangana, India

ABSTRACT

Genetic diversity of two important genes (sodium channel and USP) from intra specific populations of *Aphis gossypii* were carried out for a better understanding of sequence variations, which facilitates enumeration of multi-host versatilities in *Aphis gossypii*. Thus, upon cloning, sequencing and aligning, 23 out of 586 bp (3.92%) and 24 out of 1146bp (2.09%) nucleotide variations were chronicled for sodium channel and USP genes respectively with 2 parsimony informative sites for both the genes. Further, our study concluded that there was a narrow sequence variation observed between populations on constructing a phylogenetic tree which resulted in a single clade for both the genes. The information generated from the present study aids in future insect pest management strategy like RNA interference which is futuristic, safe and acts as sequence specific biopesticide.

KEYWORDS: Sodium Channel, Ultraspiracle Protein, RNAI, Diversity

INTRODUCTION

Of numerous sucking agricultural pests damaging commercial crop like cotton, *Aphis gossypii* (Hemiptera) is paramount in its spectrum. This is because of its wide-reaching distribution with multi-host range, parthenogenetic reproduction, polyphenism, season dependent host modification and vectoring abilities (Blackman et al., 1984; Lecalnt et al., 1994; Hogenhout SA et al., 2008). Development of multi-host capabilities may be due to the selection of adaptive characters and genetic variations in insect populations (Rice 1987; Diehl and Bush 1989). Sometimes the polyphagous insects may either adopt monophagous or oligophagous feeding modes in a micro-ecological region (Karowe 1989). Thus, selective mode of feeding leads in specialization and adoptions to different ecological conditions (Berenbaum 1996; Kaweck 1997). The cause for adaptive versatility may be due to the variations in the genetic makeup, thus making *A. gossypii* a notorious polyphagous pest.

Presently, control strategies rely upon either overuse of insecticides viz. organophosphates, DDT, endosulfan, carbamates etc., or its combinations which resulted in insecticidal resistance (Kung et al., 1961; Villatte et al., 1999; Wei et al., 1988; Zhang et al., 1997; Ahmad et al., 1999; Herron et al., 2001; Delorme et al., 1997; Furk et al., 1980; Bobert et al., 1994). Though, globally 10% of insecticides were used in management of cotton alone, annual crop damages due to aphids was estimated at hundreds of millions of dollars (Blackman et al., 1984; Oerke et al., 1994; Morrison et al., 1998). In India, approximately 50% of total insecticide worth of 600 Million US $ were spent for cultivating cotton, which shares only 5 % of cropping land (Ghosh, 2001).

The need of the hour is to facilitate effective futuristic technology viz. ribonucleic acid interference (RNAi), which is eco-friendly and an alternative in managing agricultural pests like aphids. RNAi is highly sequence specific in nature so that it can be harnessed as species specific bio pesticide (Caplen et al., 2000; Baum et al., 2007; Price et al., 2008; Whyard et al., 2009; Mao et al., 2007).

Understanding the genetic differences among geographically distinct intraspecific populations is a pre-requisite in studying their dynamics, selection pressures to various behaviour and responses and also in designing a species specific bio pesticide for its management. In the present study, we have successfully cloned and sequenced the sodium channelandultraspiracle protein (USP) genes from *A. gossypii* collected across 12regions of Karnataka, India and constructed a *NJ* tree for analysing the genetic diversity.

MATERIALS AND METHODS

Specimen Collection

Apterous forms of *A. gossypii* were collected into RNA-stabilizing solution (*RNA later*®- Ambion, USA) from 12 locations and from the cotton host (*Gossypiumhirsutum*) (Table 2). Tubes containing specimens were conveyed on ice packs in a closed thermos cool container and stored at -80 ºC until subsequent molecular studies.

Total RNA Extraction and RT-PCR

RNAlater was blotted from the specimens before RNA extraction. Total RNA was extracted from *A. gossypii* specimen using MACHEREY-NAGAL GmbH kit (Germany) following the standard protocol. The quality of the RNA was estimated with the aid of NanoDropLite (Thermo Scientific, Germany) and stored at -20ºC until further molecular usage. Primers specific to sodium channel and USP genes were designed by using nucleotide sequences that were earlier submitted with NCBI-GenBank for *A. gossypii*, with the aid of Integrated DNA technologies online software.

RNase free DNase (Fermentas Life Sciences, USA) was used to remove the DNA contamination from total RNA. Complementary DNA was synthesized by using Reverse transcriptase enzyme as per manufacturer's protocol (Fermentas Life Sciences, Germany). Coding sequences were amplified from the first strand cDNA using gene specific primer (Table 1) under the following conditions: 10X reaction buffer; (10mM) dNTP mix; 10 Picomoles of both Forward and reverse primers (Table 1) respectively for individual gene; 1 U of Taq DNA Polymerase (TaKaRa, Canada); (1:3) diluted cDNA and finally nuclease free water making up the reaction volume to 50.0 µl. Invitro amplification was carried out in a thermal cycler (Veriti 96 wells, AB-Applied Biosystems, USA) bearing the temperature cycles as: 4 minutes at 94ºC as initial denaturation; followed by 35 cycles at 94ºC for 40 sec; primer annealing for 40 sec (sodium channel 58ºC and USP 55ºC respectively); strand extension for 45 sec at 72ºC and final extension for 10 minutes at 72ºC. The PCR amplicons were resolved in 1.0% agarose gel stained with 10µg/ml ethidium bromide (intercalating dye) and visualised under UV transilluminator (UVP, Bio-Rad, UK) for verifying the product size.

Molecular Cloning and Sequencing of Candidate Genes

After documenting the gel, it was excised and purified by using Nucleospin extract II kit (Macherey Nagel, Germany) as per the manufacturer's recommendations. The eluted amplicons were ligated onto a general purpose cloning vector (PTZ 57R/T) (Fermentas, GmbH, Germany) and transformed into *Escherichia coli* (DH5α strain) as per manufacturer's instructions. Blue white colony screening was carried out and plasmids were isolated using GenJet™ Plasmid miniPrep kit (Fermentas Life sciences, UK) from the overnight inoculated positive clones maintained in LB broth.

Sequencing was carried out by using universal M13 primers both in forward and reverse directions in an automated sequencer (ABI Prism® 3730 XL DNA Analyser) (Xcelris Labs, Ahmadabad, India).

Sequence Analysis

Homology search for the candidate gene sequences were carried out by using NCBI-BLAST (http://www.ncbi.nlm.nih.gov). Further, gene sequences were aligned by an alignment tool Bioedit (V.7.0.9.0) (Hall, 1999) and MEGA. 5.0 (Tamura et al., 2011) for determining sequence variations. Neighbour-Joining tress (*NJ* tree) were constructed with the aid of K2P (Kimura-2-parameter) distance model (Kimura, 1980; Saitou and Nei, 1987).

RESULTS

Divergence Analysis

In the present study, we have successfully cloned and sequencedtwo genes (sodium channel and USP) from 12 intraspecific populations of *A. gossypii* (Table 2). All the gene sequences generated in the present study were deposited in NCBI-GenBank repository (Table 2). Nucleotide variations for each gene were analysed by sequence alignment tool, Bioedit 7.2.5. Accordingly, a total of 23 nucleotides out of 586 bp (3.92%) and 24 out of 1146bp (2.09%) variations were observed from sodium channel and USP genes respectively. The sequence analysis carried out in MEGA 6.0 resulted in 2 parsimony informative (Pi) sites for both the genes. Nucleotide frequencies chronicled for sodium channeland USP genes were 28.36% (A), 31.90% (T), 18.90% (C), 20.85% (G) and 31.83% (A), 27.66% (T), 19.85% (C), 20.66% (G) with observed maximum composite likelihood (MCL) Transition/Transversion bias (R) being 0.984 and 3.041 respectively. Upon deducing amino acid from cDNA sequences for thesodium channel and USP genes, 586 and 1146bp nucleotide sequence encoded 195 and 382 amino acid respectively.

NJ Analysis

The phylogenetic tree was constructed based on nucleotide sequences of both sodium channel and USP genes implementing MEGA. V.6.0 (Tamura, 2013) through Neighbor-Joining model (Saitou et al., 1987) with 1000 Bootstrap replicates (Felsenstein, 1985). The phylogenetic tree showed that all the 12 populations grouped into a single cluster, establishing a narrow nucleotide variation for both the genes (Fig. A and B).

DISCUSSIONS

Enumerating genetic diversity forms a paramount prerequisite in assessing nature, selection forces, dynamics of a population and also their management (Nei 1987; Pamilo 1984; Fakrudin et al., 2004). The extent of genetic variation is reliant upon various facts viz. host spectrum, gene flow across populations and the period since the separation (Templeton et al., 1990). The present study is based on genetic variations among candidate genes of *A. gossypii* population under study. The study uncovered the nucleotide sequence variations of the selected candidate genes, had a narrow variability within the population. This can be strongly deciphered by forming a single clade on constructing a phylogenetic tree with nucleotide variations. Thus, understanding genetic variation within a demographic population will not merely fetch information on population studies but, even facilitates in management strategies like RNA interference (RNAi). Since RNAi is a sequence-specific mechanism (Dykxhoorn DM, Novina CD, 2003), double stranded RNA specific to candidate genes can be successfully used in all population of *A. gossypii* under study for gene silencing purposes. Because the sequences of both the genes have a narrow intraspecific variation, RNAi can be implanted as an effective management strategy in controlling

the outburst of *A. gossypii*.

The candidate gene viz. sodium channel plays a crucial role in the propagation of action potential across neuronal and in excited cells. Unlike mammalians, which comprises of 9 sodium channel genes with α and ß-subunits (Goldin, 2002), insect counterparts with only one sodium channel gene (Dong, 2010). Because of their profound functions in excitable and neuronal cells, they are elected as potential targets in pest management.

Sodium channel gene was earlier targeted with the aid of neurotoxins extracted from plants and animals (Catterall et al., 2007) and synthetic insecticides like pyrethroids (Wang et al., 2003). Extensive use of these synthetic insecticides led in control failures, resultingin resistance gain. As a result, insect pose insensitivity against these chemical insecticides forming knock-down Resistance (Ingles et al., 1996; Willamsson et al., 1996). Thus, silencing sodium channel gene would disturb the normal functioning of action potentials along the neuron.

CONCLUSIONS

While another gene, ultraspiracle protein (USP) belongs to group II nuclear receptor family, which aids transcription. USP is diversified across metazoans species ranging from sponges to mammals (Wiens et al., 2003). USP heterodimerize whenever bound by another ligand-dependent transcription factor viz. Ecdysteroid receptor hormone (EcR) forming EcRcomplex (Oro et al., 1990;YAO et al., 1992;1993). The ECR complex in conjunction with Juvenile hormone plays a pivotal role such as development and reproduction in arthropods (Baldwin et al., 2001; Mu et al., 2002). Targeting this gene would imbalance the normal homology of the insect hormone metabolism, thereby controlling the pest.

Thus, the present study on the genetic diversity of sodium channel and ultraspiracle protein will be the groundwork for future aspect on RNAi, which is a safe and futuristic mechanism in managing sucking pests like *A. gossypii*.

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APPENDICES

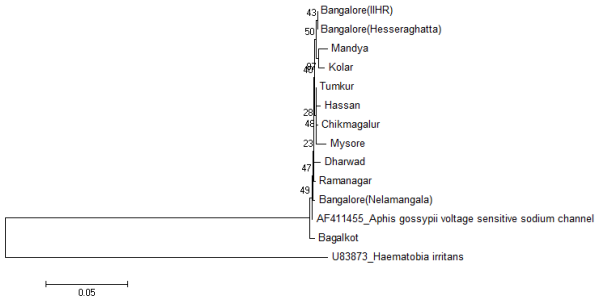
Table 1: Primers Adopted for CDNA Synthesis of Sodium Channel and Ultraspiracleprotein Genes

Gene	Application	Primer Sequence (5′-3′)	Product size (BP)
sodium channel	PCR	F:GCTAAATCGTGGCCCACACTT R:TCATCTCTACTGGTTCCTTAG	586
ultraspiracle protein	PCR	F:ATGGGTCCTCAGTCACCTCTA R: TCATGTAGCTACTTGAACGTC	1146

Table 2: A. GOSSYPII Sample Details with Accession Numbers

Gene	Location	NCBI Accession Number
sodium channel	Bangalore (IIHR)	KT365907
	Hesarghatta	KT715822
	Nelamangala	KT715823
	Tumkur	KT715824
	Hassan	KT715825
	Chikmagalur	KT715826
	Mysore	KT715827
	Mandya	KT715828
	Ramanagara	KT715829

	Kolar Dharwad Bagalkot	KT715830 KT715831 KT715832
ultraspiracle protein	Bangalore (IIHR) Hesarghatta Nelamangala Tumkur Hassan Chikmagalur Mysore Mandya Ramanagara Kolar Dharwad Bagalkot	KT365906 KT725432 KT725433 KT725434 KT725435 KT725436 KT725437 KT725438 KT725439 KT725440 KT725441 KT725442



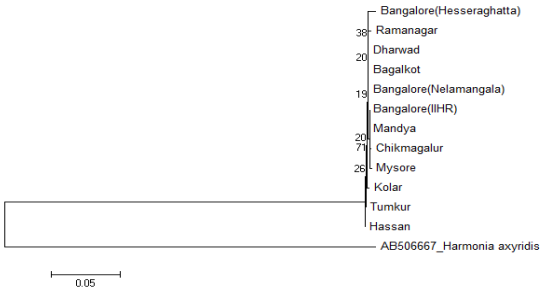


Figure.1a **Figure 1b**

Figure 1: Phylogenetic Trees for *A. Gossypii* Genes Based on Nucleotide Sequences Using MEGA 6.06. (NJ Method with Bootstrap 1000 Replicates) Figure 1a: Voltage Sensitive Sodium Channel Figure 1b: Ultraspiracle Protein With Outgroup *Haematobiairritans* (U83873) and *Harmonia Axyridis* (AB506667) Respectively

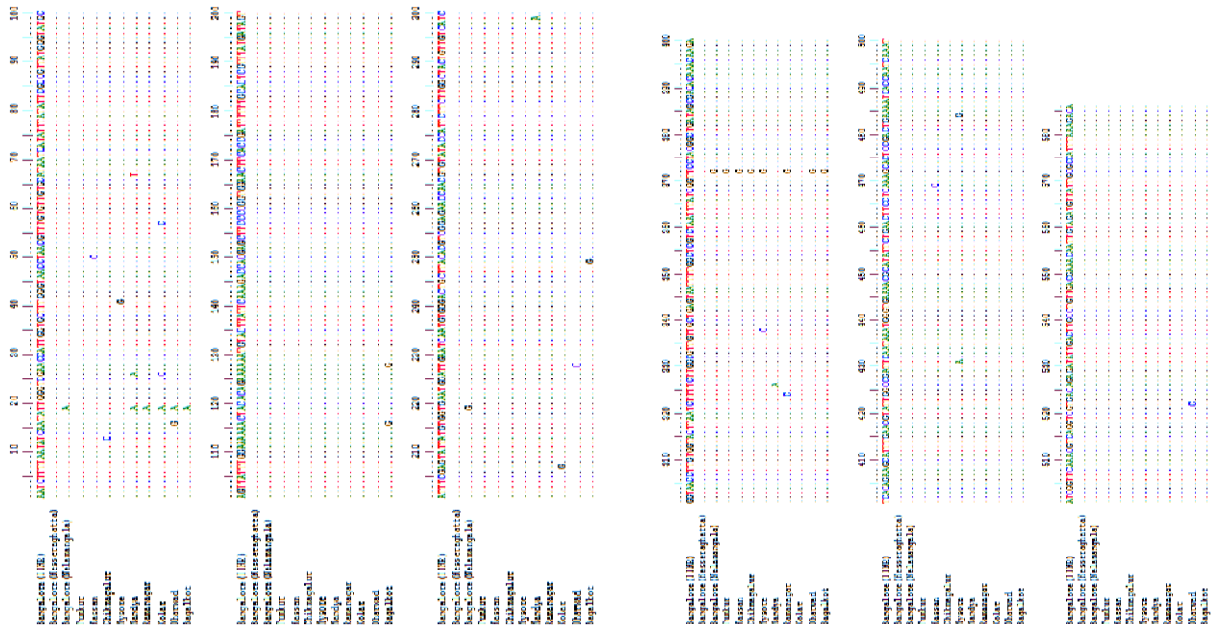


Figure 2a

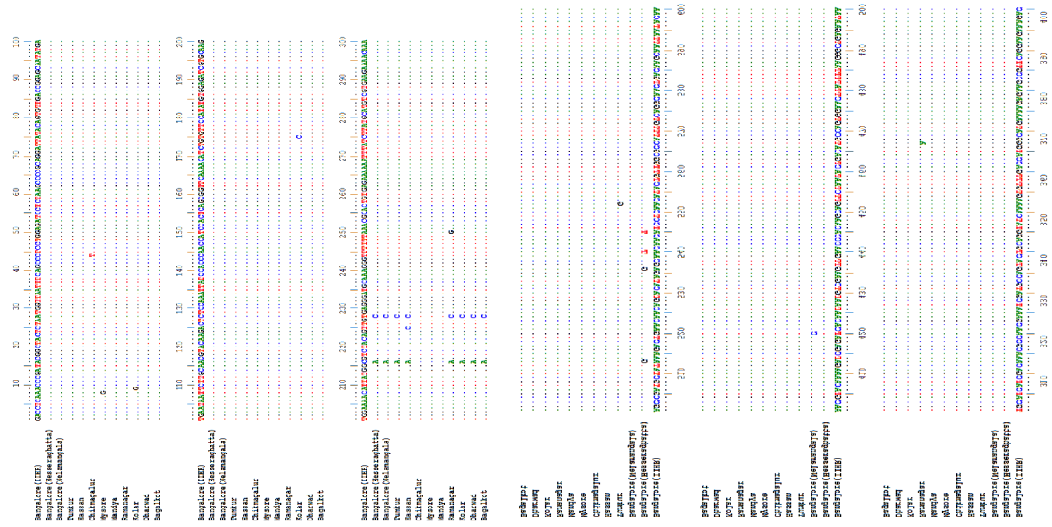


Figure 2b

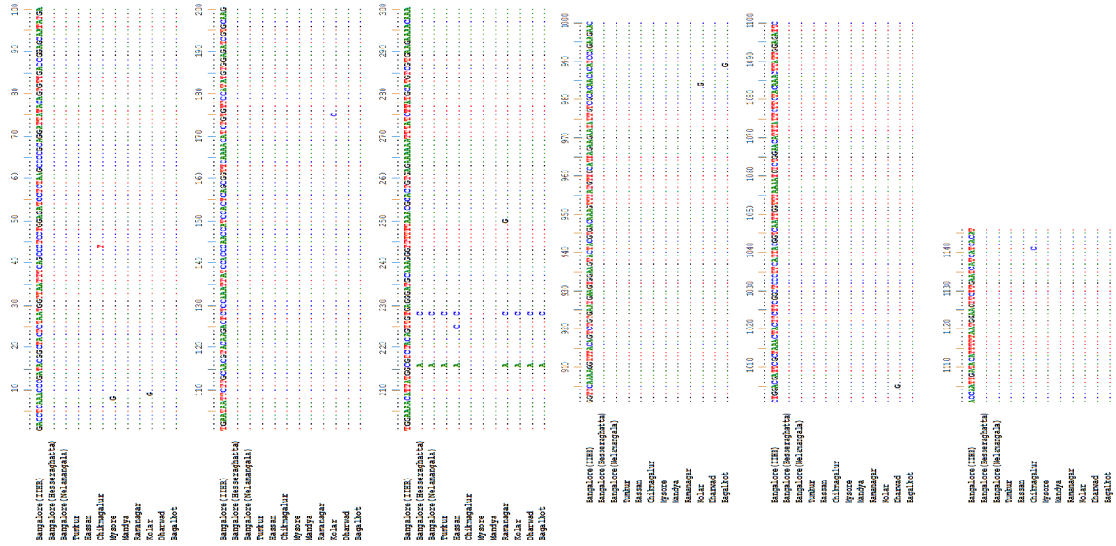


Figure 2b Contd

Figure 2: Comparisons of Nucleotide Sequences for *A. Gossypii* Genes Using Bioedit 7.2.5

Figure 2a: Voltage Sensitive Sodium Channel (586 Bp) Figure 2b: Ultraspiracle Protein (1146 Bp)